ISOLATION AND CHARACTERIZATION OF THREE NOVEL POLYETHER ANTIBIOTICS AND THREE NOVEL ACTINOMYCINS AS COMETABOLITES OF THE SAME *STREPTOMYCES* SP. X-14873, ATCC 31679

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Streptomyces sp. X-14873 (ATCC 31679) has been found to produce a number of secondary metabolites. Three have been identified as the novel actinomycins, X-14873B, C and D, each of which contains both proline and 3-hydroxy-5-methylproline. Potentially, the most important microbial product from this fermentation is the novel polyether antibiotic X-14873A (**1**) which differs from lysocellin (**5**) only in the substituents at carbons C-4 and C-5 in the tetrahydropyranyl ring. The methyl group and proton in lysocellin are replaced by an ethyl and hydroxyl group, respectively in X-14873A. In addition, two other novel polyethers, X-14873H (**2**) and G (**3**), were isolated and shown to differ from **1** in lacking a carboxyl group and in the case of **3**, possessing an ether bridge across the terminal tetrahydrofuranyl ring system.

In 1982, a polyether antibiotic complex with coccidiostat activity superior to any other antibiotic of this class was first reported[1]. In the following year, the biological and ionophorous properties of these potent coccidiostats, X-14868A, B, C and D were published[2] in this journal. Subsequently, X-14868A was renamed maduramycin in a communication[3] on the ^{13}C NMR spectroscopy of the antibiotic and a paper[4] describing the biosynthesis of maduramycins α (X-14868A) and β (X-14868C).

In this paper, a novel polyether antibiotic X-14873A is described. The major interest in this new compound arises from the marked improvement the antibiotic causes in the efficiency of feed conversion in ruminants[5,6]. In addition, two other related antibiotics, X-14873G and X-14873H and three novel actinomycins, X-14873B, C and D are reported. All six of these previously unreported microbial metabolites were isolated from the same *Streptomyces* sp. (X-14873) which has been assigned ATCC No. 31679[7].

Isolation of Actinomycins X-14873B, C and D

As part of our search for novel polyether antibiotics with exceptional activity either as coccidiostats or ruminant growth promotants[6], the whole broth from a fermentation of *Streptomyces* sp. X-14873 (ATCC 31679) was extracted with methylene chloride. In a number of partition and chromatography steps described by LIU *et al.*[5], the first metabolites to be isolated and characterized belonged to the actinomycin class of antineoplastic antibiotics. From a 475-liter fermentation, the following yields were obtained:

1.4 g actinomycin X-14873B; found: C 57.42, 57.37, H 6.98, 7.00, N 12.75, 12.67, $[\alpha]_D$ $-244.5°$ (*c* 1, MeOH), $-349.6°$ (*c* 1, $CHCl_3$).

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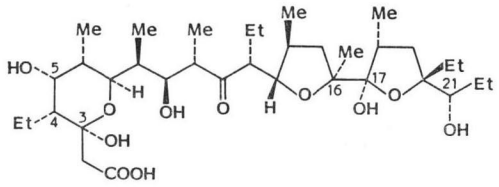
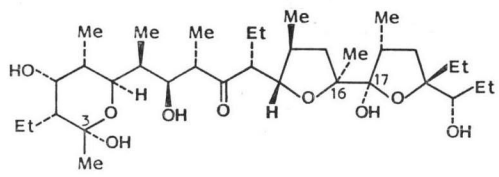
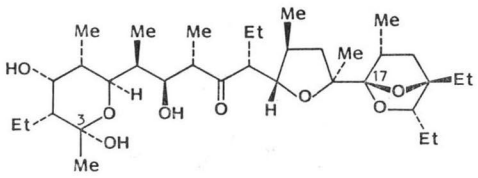
Scheme 1. The structures of antibiotics X-14873A, H, G, X-14889D, and lysocellin.



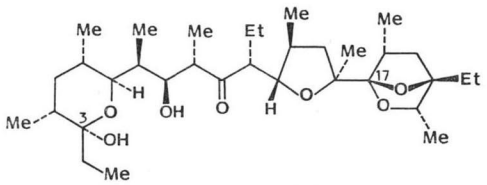
X-14873A (**1**)



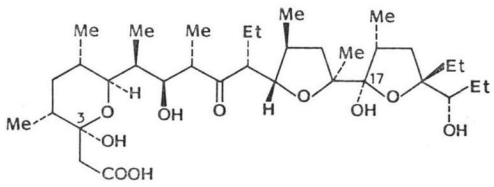
X-14873H (**2**)



X-14873G (**3**)



X-14889D (**4**)



Lysocellin (**5**)

1.2 g actinomycin X-14873C; found: C 58.12, 57.97, H 7.06, 7.01, N 13.00, 12.95, $[\alpha]_D$ −299.1° (*c* 1, MeOH), −395.2° (*c* 1, $CHCl_3$).

3.9 g actinomycin X-14873D; found: C 56.50, 56.49, H 6.80, 7.00, N 12.06, 12.16, $[\alpha]_D$ +11° (*c* 0.2, MeOH), −145.5° (*c* 1, $CHCl_3$).

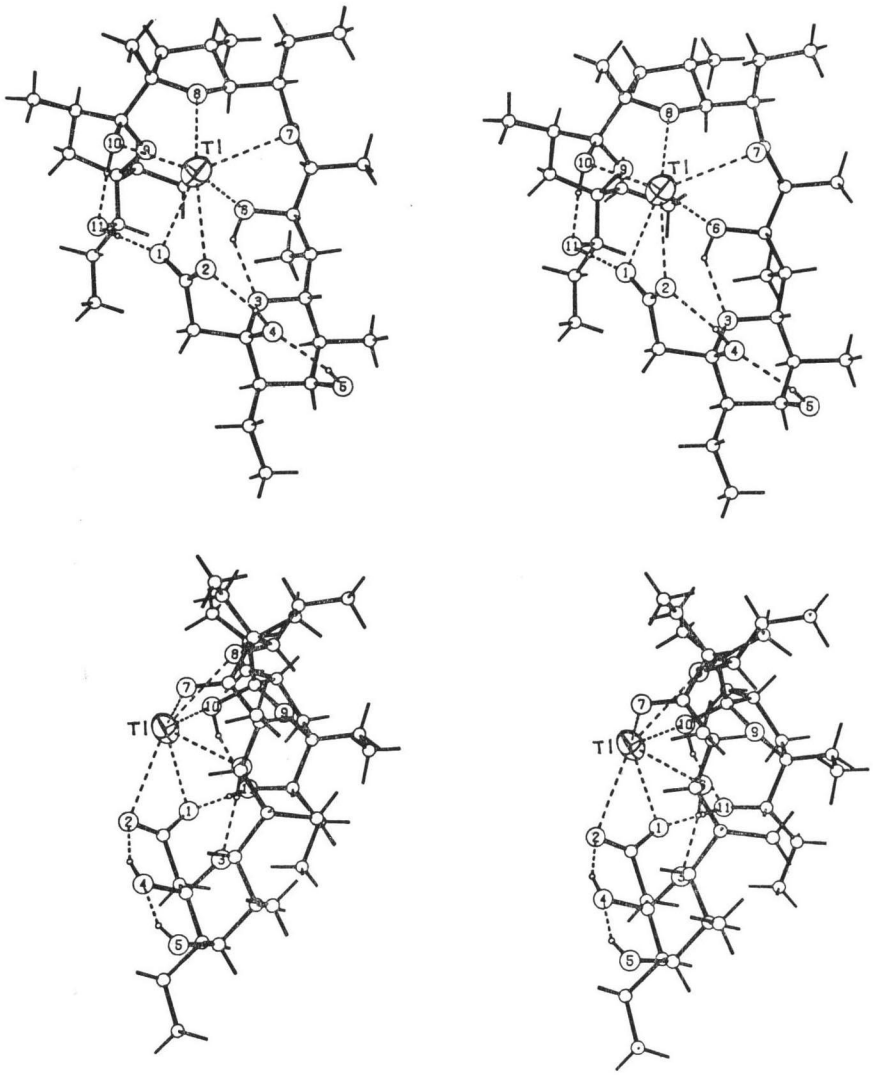
Mass spectral analysis by fast atom bombardment of actinomycins X-14873C and X-14873D showed the two antibiotics to be isomeric with a molecular formula of $C_{61}H_{86}N_{12}O_{18}$ (MW 1275.44). Actinomycin X-14873B, C and D give on hydrolysis inter alia both proline and 3-hydroxy-5-methylproline. These results clearly distinguish all three from any known actinomycin. Actinomycin X-14873B was both more toxic (LD_{50} 0.16 mg/kg ip, 2.9 mg/kg po) and more active as an antitumor agent against S-180 tumor implant in mice (ED_{50} 0.048 mg/kg) than actinomycins X-14873C and D (LD_{50} values greater than 1,000 mg/kg po and ED_{50} values in the 6~12 mg/kg range). This tremendous contrast in biological activity carried over into the coccidiostat area. Actinomycins X-14873C and D, like all other actinomycins, showed no activity, but X-14873B did exhibit *in vivo* coccidiostat activity in poultry.

Isolation of the Sodium Salt of Polyether Antibiotic X-14873 and Conversion to the Thallium form for X-Ray Analysis

From the same fermentation broth that produced the three novel actinomycins described in the previous section, fractions containing what appeared to be a novel polyether antibiotic were combined to produce 19.3 g of a crude residue which was further chromatographed on silica gel using a gradient

Fig. 1. Two stereoscopic views of the thallium salt of antibiotic X-14873A.

In the upper drawing, the thallium coordination and hydrogen bonding are clearly displayed. In the lower view the molecule has been rotated 70° about the vertical axis to illustrate the exposed nature of the thallium cation.



of methylene chloride with increasing amounts of methanol (up to 5%). The solvent was removed from the combined fractions containing the antibiotic; and the residue was crystallized from acetonitrile to yield antibiotic X-14873A, sodium salt, mp 152°C, $[\alpha]_D$ −5.4° (*c* 1, $CHCl_3$).

Calcd for $C_{35}H_{61}O_{11}Na$ (680.87): C 61.73, H 9.05, Na 3.38.
Found: C 61.08, H 9.24, Na 3.15.

A solution of the sodium salt of antibiotic X-14873A in methylene chloride was washed in turn with aqueous 1 N HCl, water, and four times with an aqueous solution of thallium hydroxide. The solvent phase was then concentrated and on addition of *n*-hexane, the crystalline thallium salt of antibiotic X-14873A was recovered, mp 154°C.

Calcd for $C_{35}H_{61}O_{11}Tl$ (862.25): C 48.76, H 7.13, Tl 23.70.
Found: C 49.01, H 7.07, Tl 21.91.

Table 1. Coordination of thallium ion by antibiotic X-14873A.

Oxygen	Distance (Å)
	Tl···O
O (1)	2.73
O (10)	2.82
O (6)	2.85
O (7)	2.90
O (2)	2.92
O (8)	2.94

Table 2. Hydrogen bonds present in the thallium salt of antibiotic X-14873A.

Hydrogen bond	Distance (Å)
	O····O
O (4)-H···O (2)	2.63
O (5)-H···O (4)	2.80
O (6)-H···O (3)	2.74
O (10)-H···O (11)	2.77
O (11)-H···O (1)	2.61

Table 3. Crystal data for the thallium salt of antibiotic X-14873A.

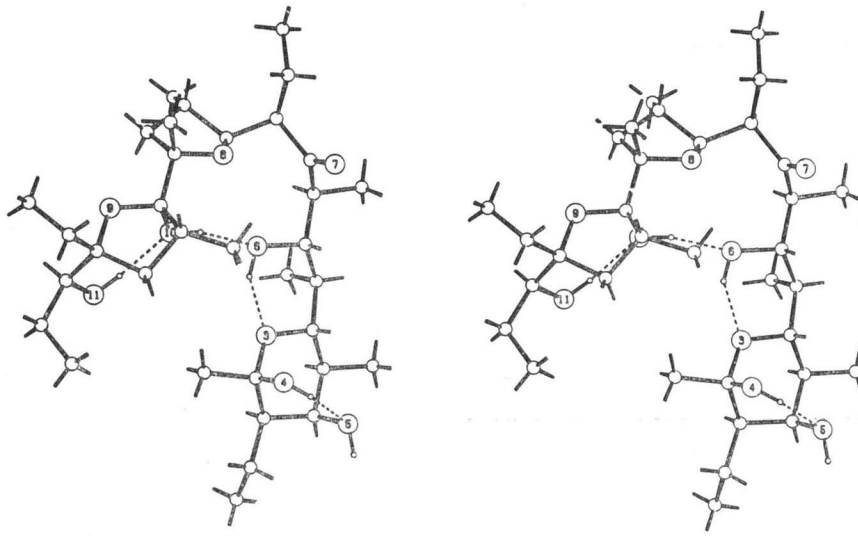
Formula	$C_{35}H_{61}O_{11}Tl$
Formula weight	862.23
Space group	$P2_1$
a	9.558 (2) A
b	10.182 (2) A
c	19.934 (4) A
β	91.51 (1)°
Z	2
d_{calcd}	1.476 g cm^{-3}
μ (CuK_α)	85.2 cm^{-1}

The structure and absolute configuration of antibiotic X-14873A (**1**) were determined by X-ray analysis of the aforementioned thallium salt. Two stereoscopic projections of the thallium salt are shown in Fig. 1. The most striking aspect of the crystal structure is the exposed nature of one side of the thallium cation. One hemisphere of the cation is coordinated with six oxygen atoms (Table 1), but the other contains neither a solvent oxygen nor a ligand from a second antibiotic molecule. The fact that there is no other oxygen atom within 3.8 Å of the thallium ion distinguishes this particular polyether complex from any other reported example[8]. Another unusual aspect of the crystalline complex is the major role played by the carboxyl group in the intramolecular bonding in determining the conformation of the complex. The carboxyl group contributes two ligands to the cation (Table 1) and participates in two of the five intra-molecular hydrogen bonds (Table 2).

The absolute stereochemistry is based on the anomalous scattering of the thallium atom ($\Delta f' = -4.72$, $\Delta f'' = 7.39$). The isotropic and anisotropic refinements (without hydrogen atoms) were done with $\Delta f'' = 0$. Two final refinements were carried out in parallel, one for $\Delta f''$ and the other for $-\Delta f''$ (equivalent to refining the antipode). The final weighted discrepancy indices were 0.0367 for $\Delta f''$ (the configuration shown in Fig. 1) and 0.0474 for $-\Delta f''$. Thus according to the test described by HAMILTON[9], the absolute configuration is conclusively established as that for $\Delta f''$.

The crystal data are summarized in Table 3. The intensity data were measured on a Hilger-Watts diffractometer (Ni-filtered $CuK\alpha$ radiation, θ-2θ scans, pulse-height discrimination). The size of the crystal used for data collection was approximately $0.20 \times 0.30 \times 0.30$ mm; the data were corrected for absorption. Of the 2799 independent reflections for $\theta < 57°$, 2734 were considered to be observed [$I > 2.5\sigma(I)$]. The structure was solved by the heavy-atom method and was refined by full-matrix least squares. In the final refinement, anisotropic thermal parameters were used for the non-hydrogen atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are $R = 0.029$ and $wR = 0.037$ for the 2734 observed reflections. The final difference map has no peaks greater than ± 0.8 eA^{-3} except for one of 1.2 eA^{-3} near the thallium ion.

Fig. 2. Stereoscopic drawing of antibiotic X-14873H.
Comparison with the upper drawing in Fig. 1 reveals the rotation of the bond between the two tetrahydrofuranyl rings on going from antibiotic X-14873A to X-14873H.



Isolation of Polyether Antibiotics X-14873G and X-14873H

The whole broth from a later fermentation of *Streptomyces* X-14873 was extracted with ethyl acetate and the extract treated as for the earlier methylene chloride extract. On concentration of the solvent phase, crude antibiotic X-14873A crystallized out. The mother liquor was concentrated under reduced pressure and chromatographed on silica gel using gradients consisting of different mixtures of methylene chloride, hexane and acetone[5]. Fractions were pooled according to their TLC homogeneity. The first pooly yielded antibiotic X-14873G, which was crystallized from diethyl ether - hexane, mp 152~153°C, $[\alpha]_D$ +5.5° (*c* 1, $CHCl_3$).

Calcd for $C_{34}H_{60}O_8$ (596.82): C 68.42, H 10.13.
Found: C 68.28, H 10.10.

A later set of fractions were pooled to yield antibiotic X-14873H. Crystallization from methylene chloride - hexane gave X-14873H, mp 145~146°C, $[\alpha]_D$ −5.3° (*c* 1, $CHCl_3$), +3.6° (*c* 1, MeOH).

Calcd for $C_{34}H_{62}O_9$ (614.84): C 66.41, H 10.16.
Found: C 66.43, 66.55, H 9.92, 9.95.

Table 4. Hydrogen bonds in antibiotic X-14873H.

Hydrogen bond	Distance (Å)
	O···O
O (4)-H···O (5)	2.72
O (5)-H···O (11)′	2.77
O (6)-H···O (3)	2.61
O (10)-H···O (6)	2.78
O (11)-H···O (10)	2.78

O (11)′ belongs to a neighboring molecule.

X-Ray Analysis of Antibiotic X-14873H

The structure and absolute configuration of antibiotic X-14873H (**2**) were determined by single-crystal X-ray analysis. A stereoscopic drawing of a molecule of the antibiotic is shown in Fig. 2. In the case of antibiotic X-14873H, the most striking feature of the structure is the lack of a carboxyl group which immediately explains the inability of the compound to form salts. Of the five hydroxyls

Table 5. Torsion angles (°) with standard deviations in parentheses.

	X-14873A Tl salt	X-14873H
C(1)-C(2)-C(3)-O(3)	−60.5 (9)	
C(2)-C(3)-O(3)-C(7)	177.1 (6)	177.6 (6)
C(3)-O(3)-C(7)-C(8)	−170.6 (6)	−174.0 (6)
O(3)-C(7)-C(8)-C(9)	55.7 (8)	58.4 (7)
C(7)-C(8)-C(9)-C(10)	159.3 (6)	172.7 (6)
C(8)-C(9)-C(10)-C(11)	−177.6 (6)	−169.3 (6)
C(9)-C(10)-C(11)-C(12)	−142.4 (7)	−126.2 (7)
C(10)-C(11)-C(12)-C(13)	82.8 (7)	76.5 (8)
C(11)-C(12)-C(13)-O(8)	63.7 (8)	64.4 (7)
C(12)-C(13)-O(8)-C(16)	156.4 (6)	154.6 (5)
C(13)-O(8)-C(16)-C(17)	108.2 (6)	115.3 (6)
O(8)-C(16)-C(17)-O(9)	−52.4 (8)	−169.3 (5)
C(16)-C(17)-O(9)-C(20)	−147.9 (6)	−155.0 (5)
C(17)-O(9)-C(20)-C(21)	−108.4 (6)	−118.2 (6)
O(9)-C(20)-C(21)-C(22)	−175.0 (7)	−172.3 (7)
C(20)-C(21)-C(22)-C(23)	178.6 (9)	−61.9 (11)

Table 6. Crystal data for antibiotic X-14873H.

Formula	$C_{34}H_{62}O_9$
Formula weight	614.85
Space group	$P2_12_12_1$
a	9.586 (4) A
b	18.521 (7) A
c	20.167 (5) A
Z	4
d_{calcd}	1.141 g cm^{-3}
μ (CuK$_\alpha$)	6.6 cm^{-1}

Table 7. Comparison of the ^{13}C NMR chemical shifts of carbons C-3 and C-17 in antibiotics X-14873A (**1**), X-14873H (**2**), X-14873G (**3**), X-14889D (**4**) and lysocellin (**5**).

Antibiotic	Chemical shift (ppm) in $CDCl_3$	
	C-3	C-17
X-14873A	100.1	108.1
X-14873H	100.1	107.5
X-14873G	100.0	112.4
X-14889D	99.0	112.6
Lysocellin	98.1	108.1

present in the molecule, one forms an intermolecular hydrogen bond, O(5)-H···O(11)′, while the remaining four are involved in intramolecular hydrogen bonds as summarized in Table 4.

The backbone torsion angles of the X-14873A and X-14873H molecules are compared in Table 5. Perusal of these torsional parameters points out the conformational similarity of the two structures with the distinct exception of the C(16)-C(17) bond.

The crystal data are summarized in Table 6. The intensity data were measured on a Hilger-Watts diffractometer (Ni-filtered CuKα radiation, θ-2θ scans, pulse-height discrimination). The size of the crystal used for data collection was approximately 0.20×0.30×0.65 mm; the data were not corrected for absorption. Of the 2752 independent reflections for $\theta<57°$, 2106 were considered to be observed [I σ 2.5 (I)]. The structure was solved by a multiple-solution procedure[10] and was refined by full-matrix least squares. In the final refinement, anisotropic thermal parameters were used for non-hydrogen atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are R=0.068 and wR=0.072 for the 2106 observed reflections. The final difference map has no peaks greater than $\pm$0.3 eA^{-3}.

Structure of Antibiotic X-14873G

Microanalysis and field-desorption mass spectrometry of the novel polyether antibiotic X-14873G

support the chemical formula, $C_{34}H_{60}O_8$ (MW 596.8) which implies that, in addition to the carboxyl group lost in going from antibiotic X-14873A to X-14873H, a dehydration also occurs in the further transformation to antibiotic X-14873G. The inability of antibiotic X-14873G to form salts confirmed the absence of a carboxyl group. Thus, the question remaining is where is the molecule of water that was eliminated from X-14873H.

The structure of antibiotic X-14873G (**3**) was finally elucidated by comparing the ^{13}C NMR spectra of lysocellin, antibiotics X-14873A, G and H with that of the novel unpublished antibiotic X-14889D (**4**), the structure of which has been determined by X-ray analysis[11]. The ^{13}C NMR spectrum of the closely related polyether antibiotic lysocellin (**5**) has been fully assigned by ŌTAKE *et al.*,[12].

There are many common signals for all five compounds and the complete assignments will be published elsewhere[11], but for the purpose of this paper we need only consider the two hemiketal carbons at C-3 and C-17 (Table 7). The evidence in Table 7 strongly supports a bridged ring across the terminal tetrahydrofuranyl ring as indicated in the structures of antibiotic X-14873G (**3**) and the X-ray structure determined for antibiotic X-14889D (**4**). Formally therefore, the loss of water in going from antibiotic X-14873H to antibiotic X-14873G has occurred between the hemiketal at C-17 and the secondary alcohol at C-21.

The taxonomy of the producing culture and the biological and ionophorous properties of the three polyether antibiotics, X-14873A, G and H are described in the accompanying paper[7].

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